PMNL Isolation

Coccygeal venous or arterial blood samples (~120 mL) were collected in vacutainer tubes containing acid citrate dextrose (ACD Solution A; Fisher Scientific) on days -14 (±2) and +7 relative to parturition (~0700 h). Samples were placed on ice until PMNL isolation, which took place within 30 min of collection. Complete details of blood processing can be found in Zhou et al. (2015); in brief, PMNL were isolated from whole blood by sample centrifugation, cell lysing, and several rounds of centrifugation with PBS washing. Isolated PMNL were homogenized at full speed in a solution of 2 mL TRIzol reagent (Invitrogen, Carlsbad, CA) with 1 µL linear acrylamide (Ambion, Inc., Austin, TX). Homogenate was stored at -80ºC in RNA-free microcentrifuge tubes (Fisher Scientific, Pittsburgh, PA) for further analysis.

RNA Isolation

Once samples were thawed and centrifuged, total RNA was separated with chloroform and acid phenol:chloroform (Ambion, Inc., Austin, TX). Total RNA was precipitated with isopropanol and cleaned with 75% ethanol. Resuspension in RNA storage buffer (Ambion, Inc., Austin, TX) allowed RNA to be stored at -80ºC. Prior to storage, RNA purity was confirmed using a NanoDrop ND-1000 (NanoDrop Technologies, Rockland, DE) OD_{260mm}/OD_{280mm} ratio, and RNA quality was recorded using a 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA) RNA integrity number (**RIN**). Average RIN was 7.7 ± 0.2.

cRNA Synthesis and Labeling. The microarray platform for the present study used 4x44k-Agilent Bovine (V2) Gene Expression Microarray chips (Agilent Technologies; cat# G2519F-023647). Labeling and hybridization protocols were followed as directed by Agilent Technologies, and are also outlined in previous publications from this group (Shahzad et al., 2015). cDNA was created from 200 ng total quantity of RNA, then reverse-transcribed into Cy-

3 or Cy-5 fluorescent dye-labeled cRNA using a Low-Input Quick Amp Labeling Kit (two colors; Agilent Technologies; cat# 5190-2306). Resulting cRNA was purified using RNeasy mini spin columns (Qiagen; cat# 74104), and eluted in nuclease-free water. Yield of at least 825 ng and specific activity ≥ 6.0 pmol/µg were confirmed using the NanoDrop ND-1000 (NanoDrop Technologies). Samples were stored at -80ºC until hybridization.

Fragmentation, Hybridization, and Slide Scanning. After thawing cRNA, 825 ng of one Cy-3-labeled sample and 825 ng of one Cy-5-labeled sample were mixed together and with 11 µL of 10X Blocking Agent (Agilent Technologies; cat# 5188-5281) and 2.2 µL of 25X Fragmentation Buffer (Agilent Technologies; cat# 5185-5974). Nuclease-free water was added to bring the final volume of solution to 55 µL. Solution was placed in a 60ºC water bath for 30 min, then 55 µL of 2X GEx Hybridization Buffer (Agilent Technologies; cat# 5190-0403) was added to stop fragmentation reactions. The total solution of 110 µL was loaded onto a Hybridization Gasket Slide (Agilent Technologies; cat# G2534-60011), and hybridized to a microarray slide for 17 h in a 65ºC hybridization oven. Slides were washed as directed by Agilent Technologies, then scanned immediately in a GenePix 4000B scanner (Axon Instruments, Inc., CA) using GenePix Pro v.6.1 software. After scanning, microarray images were edited to flag low-quality spots as “bad.” These spots were excluded from slide analysis.